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Quantitative Assessment of the Effects of Metals on Microbial Degradation of Organic Chemicals

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Biodegradation inhibition of a benchmark chemical, 2,4-dichloro-phenoxyacetic acid methyl ester (2,4-DME), was used to quantify the inhibitory effects of heavy metals on aerobic microbial degradation rates of organic chemicals. This procedure used lake sediments and aufwuchs (floating mats) collected in the field or from laboratory microcosms. Effects of $CuCl_2$, $HgCl_2$, $ZnCl_2$, $Cd(NO_3)_2$, and $Cr(NO_3)_3$ at initial concentrations ranging from 0.3 μM to 73 mM (approximately 0.1 to 10,000 mg liter^{-1}) were investigated. In general, such metallic compounds appeared to be considerably more inhibitory to the biodegradation of an organic chemical than high concentrations of microbially toxic organics studied previously. Effects of various metal concentrations were evaluated based on the following: (i) estimated MICs, (ii) concentrations that caused a significant effect on biodegradation parameters (both a >10% decrease in V_{max} and a >10% increase in $t_{1/2}$ for 2,4-DME degradation), and (iii) concentrations that caused biodegradation half-life doublings (HLDs). The MICs of metals in sediment were lowest for Zn^{2+} (0.10 μM) and highest for Cd^{2+} and Cu^{2+} (0.9 and 1.2 μM, respectively). The MICs of metals in aufwuchs were lowest for Hg^{2+} (0.01 μM), intermediate for Cu^{2+} and Zn^{2+} (0.42 and 0.62 μM, respectively), and highest for Cr^{3+} and Cd^{2+} (3.4 and 5.6 μM, respectively). Compared with Cu^{2+} on aufwuchs, 70 times more Zn^{2+}, 250 times more Cr^{3+}, and 1,000 times more Cd^{2+} was required to significantly affect aufwuchs biodegradation rate parameters and coefficients (V_{max} and $t_{1/2}$). Aufwuchs was significantly affected by the lowest Hg^{2+} concentration tested (5 μM). The HLDs of metals in sediment were 0.17, 0.24, and 0.79 mM for Cu^{2+}, Cd^{2+}, and Zn^{2+}, respectively. The HLDs of metals in aufwuchs were 2.0 and 3.0 μM for Cu^{2+} and Hg^{2+}, respectively, and 0.14, 0.44, and 1.2 mM for Cr^{3+}, Zn^{2+}, and Cd^{2+}, respectively.

Toxic metals are common contaminants of natural waters (6, 15, 16) and may adversely affect potentially important biodegradation processes occurring in the environment. Sources of these pollutants may include leachates from hazardous waste sites, discharges from industrial plants, and effluents from wastewater treatment plants. Chemical wastes at land disposal sites may contain toxic metals in particularly high concentrations. Numerous research efforts have been directed toward determining safe levels of these pollutants that would not adversely affect biota in the environment. Some methods developed for these safety determinations have incorporated axenic cultures isolated from environmental samples and grown in defined medium (3, 10). Such methods are limited in their application to field situations, however, because very rarely, if ever, are conditions in nature such that only one microbial species is active or are conditions in nature comparable with the conditions on defined laboratory media. Other studies have focused on lethal aspects of toxic metals, that is, the determinations of concentrations of pollutants that kill biota or totally inhibit the reproductive potential of organisms. These studies typically rely on physiologically restrictive processes, i.e., sulfate reduction, methanogenesis (4, 9), acetate incorporation, [^{14}C]glucose uptake (1), and more recently, [^{3}H]thymidine incorporation (2, 8, 23), all of which may not relate to organic chemical biodegradation rates. Acute toxicity is generally expressed in these studies (e.g., 50% lethal concentration, 50% effective concentration).

The objective of this work was to develop and test a more direct and quantitative measure of the inhibitory effects of metals on the aerobic microbial degradation of organic chemicals. Data presented regarding the inhibitory effects of metals on biodegradation rates exemplify the results obtained with the method; they are not intended to fully elucidate the effects of the metals studied. Our approach to predicting inhibitory levels of metals involved measuring the rates at which a benchmark chemical was biodegraded under a wide range of metal ion concentrations. Five of the eight heavy metals targeted for concern in the U.S. Environmental Protection Agency's priority list of pollutants (21)—cadmium, chromium, copper, mercury, and zinc—were chosen for use in these studies. 2,4-Dichloro-phenoxyacetic acid methyl ester (2,4-DME) was used as the benchmark organic chemical because considerable data concerning its microbial degradation kinetics in a variety of laboratory and field systems are available (11, 17) and because it is rapidly degraded by a wide variety of microbes, including bacteria, fungi, and algae.

This method, like other procedures currently used in making environmental risk assessments, is designed with various considerations of practicality in mind, i.e., cost of obtaining data, sophistication of the instrumentation required, and degree of skills needed for those involved in making the risk assessment decisions. The most scientifically rigorous methods may require data obtainable only with highly sophisticated instrumentation and may necessitate a degree of skill in the user not ordinarily possessed by those involved in making environmental risk assessments. Because of this, a particular method may be the most

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scientifically sound one possible but in practice be unusable. On the other hand, a method might require data that can be easily obtained or estimated and only modest technical skills of the user. In the absence of better tools, such a method may be widely used although it is largely indefensible with respect to its scientific merit. Reaching the goal of providing a method that is both adequately sound from a scientific standpoint (so that one can at least determine and accept its inherent degrees of error) and sufficiently user friendly (so that it will be widely utilized by those involved in making risk assessments) is an exercise in compromise. Therefore, developing such methods may be an endeavor in which the scientist neither completely satisfies those who are concerned with scientific credibility nor those who wrestle with the practical utility of models. Consequently, the method presented here is one that, depending on the analytical capabilities available and the technical expertise of the user, may be modified to take into consideration any of the numerous environmental factors that may influence the toxic effects of metals on biologically mediated reactions.

MATERIALS AND METHODS

Sediment samples were collected from the Whitehall Forest Lake near Athens, Georgia, by collecting the top 5 mm of sediment with a sterile spatula, placing the samples in sterile disposable plastic bags, and transferring them to the laboratory within 1 h from the time of collection. Sediment subsamples were diluted 1:100 (volume of sediment/volume of lake water) and kept well mixed with a magnetic stirrer while the slurries were subdivided. Samples (approximately 0.5 g [wet weight] ml^{-1}) of aufwuchs (floating mats of filamentous algae) were collected from a previously described laboratory aquatic microcosm (12, 13) with sterile spatulas, blended for 1 min with an electric blender, and diluted 2:100 (volume of aufwuchs slurry/volume of microcosm water).

The following metals and initial concentrations were used: Cu^{2+}, 0.5 μM to 2.3 mM (0.085 mg to 0.4 g of $CuCl_2 \cdot 2H_2O$ $liter^{-1}$); Zn^{2+}, 7.3 μM to 73 mM (1 mg to 10 g of $ZnCl_2$ $liter^{-1}$); Cr^{3+}, 0.25 μM to 25 mM [0.1 mg to 10 g of $Cr(NO_3)_3 \cdot 9H_2O$ $liter^{-1}$]; Cd^{2+}, 1 μM to 20 mM [0.31 mg to 6.2 g of $Cd(NO_3)_2 \cdot 4H_2O$ $liter^{-1}$]; and Hg^{2+}, 5.0 μM to 1.0 mM (1.4 to 270 mg of $HgCl_2$ $liter^{-1}$). Metal salts were dissolved and diluted in sterile, distilled water so that the addition of less than 1 ml of stock solution to a 100-ml microbial sample yielded the desired initial concentrations of test chemicals. Glassware was machine washed with Alcojet detergent (Alconox, Inc., New York, N.Y.). To ensure that water used for chemical stocks was not contaminated with metals, distilled water samples were routinely analyzed by atomic adsorption and found to have metal concentrations <100 times lower than the MICs for the metals used in this study.

All rate studies were carried out by adding a 2,4-DME aqueous stock solution to the microbial samples to obtain an initial concentration of 200 μg $liter^{-1}$. This low concentration was used to ensure that biodegradation rates would be first order to 2,4-DME concentration and that the biodegradation rate coefficients would be comparable. Synthesis of 2,4-DME and preparation of stock aqueous solutions have been reported previously (11, 14). By using duplicate cultures, degradation rates were followed through at least two half-lives.

Experiments were conducted by dispensing cultures into 150-ml Kimax milk dilution bottles with loose screw caps and incubating in a shaker at 25°C and 100 rpm. Using a Beckman Zeromatic SS-3 pH meter, pH values of the cultures were determined before and after the addition of metal salts. The pH values of the cultures (5.0 to 6.5) were largely determined by the activities of high concentrations of microbes. Therefore, any effects of the additions of trace quantities of metals to microbial cultures were undetected. Because pH changes may affect biodegradation rates, microbial samples were allowed to remain within the normal pH range of the environments from which they were taken.

2,4-DME degradation rate coefficients were determined from the rates of loss of the parent compound by analyzing 2,2,4-trimethyl pentane (isooctane) extracts of 5-ml samples taken at various times depending on the degradation rates. Concentrations of 2,4-DME were determined with a Tracor 222 (Southern Analytical Inc., Oakwood, Ga.) gas liquid chromatograph equipped with a ^{63}Ni detector and a column (2 m by 0.6 mm; internal diameter, 4 mm) packed with 3% OV-1 on 80/100 mesh Supelcoport (Supelco Co.). Pseudo-first-order transformation rate coefficients, k_1, were calculated from plots of the natural logarithm of substrate concentration versus time according to the integrated first-order rate equation:

$$\ln (C_t/C_0) = -k_1 \cdot t$$

where C_0 and C_t are the concentrations at time 0 and t, respectively. Half-lives were calculated as $0.693/k_1$.

To account for abiotic losses, k_1 values reported for 2,4-DME degradation were calculated by subtracting k_1 values obtained with autoclaved microbial samples from k_1 values determined using viable microbial samples. Abiotic degradation rate coefficients ranged from 0.21 to 3.0% of the corresponding values for microbial degradation rates.

Percentage reductions in microbial degradation rate coefficients were calculated on the basis of comparisons with control sediment or aufwuchs samples (unamended with the metal salts) as follows.

$$\% \text{ Reduction in } k_1 = [(\text{control } k_1 - \text{treatment } k_1)/\text{control } k_1] \times 100$$

The metal concentrations at which a doubling in 2,4-DME degradation half-lives ($t_{1/2}$) occurred were calculated from linear regressions of the percentages of reductions in k_1 versus logarithms of metal salt concentrations, i.e., a 50% reduction in k_1 equaled one doubling of $t_{1/2}$. These values were referred to as half-life doubling concentrations (HLDs).

Studies involving microbial degradation often present data in terms of the Michaelis-Menten values for maximum degradation rates, V_{max}. These kinetic parameters were determined using Lineweaver-Burke plots and reported for comparison with HLDs. Because degradation rates observed in our experiments were reasonably described by first-order kinetics and because comparisons of V_{max} were made only among values determined over similar ranges of 2,4-DME concentrations, $t_{1/2}$ and V_{max} were considered appropriate expressions of the data.

RESULTS AND DISCUSSION

The effects of the addition of several concentrations of the metal salts upon maximum degradation rate constants (V_{max}) and half-lives ($t_{1/2}$) of the benchmark substrate (2,4-DME) are presented in Table 1. These effects were evaluated in several ways, including (i) MICs, which were determined from abscissa intercepts of plots of percent reduction in pseudo-first-order rate coefficients (k_1) versus logarithmic

TABLE 1. Effects of metal cations on the maximum utilization rates for natural and laboratory microbiota and on the half-lives of 2,4-DME from two experiments

Cation[a], microbiota, and cation concn (mM)	V_{max} (μg liter^{-1} min^{-1}) (mean ± SD)		Relative V_{max}[b]	$t_{1/2}$ (min) (mean ± SD)		Relative $t_{1/2}$[b]
	Untreated slurry	Treated slurry		Untreated slurry	Treated slurry	
Cu^{2+}						
Aufwuchs						
0.0005[c]	0.97 ± 0.08	0.85 ± 0.01	0.88	62 ± 0.83	66 ± 2.8	1.1
0.001		0.65 ± 0.06	0.76		82 ± 3.7	1.3
0.005		0.27	0.32		320	5.2
0.01		0.10 ± 0.02	0.10		500 ± 27	8.1
0.05		0.12 ± 0.03	0.12		790 ± 160	13
Sediment						
0.003[c]	1.2	1.4 ± 0.10	1.1	28	30 ± 3.0	1.1
0.006[c]		1.1 ± 0.14	0.94		35 ± 0.90	1.3
0.03[c]		1.1 ± 0.15	0.94		40 ± 0.59	1.4
0.06		0.74 ± 0.01	0.61		46 ± 2.0	1.6
0.60		0.62 ± 0.01	0.51		71 ± 1.0	2.5
1.2		0.68 ± 0.03	0.56		97 ± 13	3.5
2.3		0.57 ± 0.11	0.47		120 ± 3.4	4.2
Zn^{2+}						
Aufwuchs						
0.007[c]	1.4 ± 0.16	1.6 ± 0.20	1.2	30 ± 1.2	32 ± 0.50	1.1
0.073		0.88 ± 0.06	0.65		54 ± 0.95	1.8
0.73		0.58 ± 0.08	0.43		89 ± 0.19	3.0
7.3		0.64 ± 0.09	0.47		89 ± 0.02	3.0
73.0		0.39 ± 0.05	0.29		170 ± 5.0	5.6
Sediment						
0.007[c]	1.8	2.2 ± 0.06	1.2	34	48 ± 5.7	1.4
0.73		0.92 ± 0.12	0.50		71 ± 0.40	2.1
3.7		0.85 ± 0.16	0.46		75 ± 1.5	2.2
15		0.46 ± 0.05	0.25		79 ± 1.6	2.3
73		0.61 ± 0.08	0.33		140 ± 4.9	4.1
Hg^{2+}						
Aufwuchs						
0.005	1.7 ± 0.18	1.1 ± 0.02	0.67	18 ± 0.39	38 ± 2.2	2.2
0.05		0.96 ± 0.04	0.86		71 ± 3.4	4.0
0.10		0.45 ± 0.27	0.40		190 ± 18	11
1.0		0.18	0.11		450	26
Cr^{3+}						
Aufwuchs						
0.0003[c]	1.2 ± 0.01	1.2 ± 0.01	1.1	32 ± 0.28	31 ± 0.60	0.96
0.003[c]		1.2 ± 0.07	0.99		32 ± 2.4	1.0
0.025[c]		1.1 ± 0.04	0.90		34 ± 1.5	1.1
0.25		0.79 ± 0.01	0.67		85 ± 1.6	2.7
2.5		0.15 ± 0.01	0.13		380 ± 19	12
25.0		0.06 ± 0.01	0.05		1,400 ± 260	420
Cd^{2+}						
Aufwuchs						
0.001[c]	0.61 ± 0.14	0.66 ± 0.18	1.1	91 ± 1.2	85 ± 0.89	0.94
0.01[c]		0.61 ± 0.03	1.0		97 ± 1.2	1.1
0.1[c]		0.74 ± 0.00	1.2		120 ± 2.4	1.3
1.0		0.51 ± 0.06	0.84		180 ± 3.8	2.0
10.0		0.29	0.48		300	3.3
Sediment						
0.005[c]	0.81	0.74 ± 0.03	0.91	65	81 ± 0.05	1.2
0.01		0.67 ± 0.08	0.82		83 ± 0.46	1.3
0.1[c]		0.76 ± 0.15	0.93		91 ± 1.5	1.4
1.0		0.27 ± 0.02	0.33		220 ± 5.8	3.3
10.0		0.28 ± 0.04	0.35		340 ± 3.6	5.2
20.0		0.36 ± 0.05	0.44		480 ± 1.3	7.3

[a] Cu^{2+}, Hg^{2+}, and Zn^{2+} added as chloride; Cr^{3+} and Cd^{2+} added as nitrate.
[b] Value for metal-treated culture divided by value for untreated culture.
[c] Did not cause both a >10% decrease in V_{max} and a >10% increase in $t_{1/2}$.

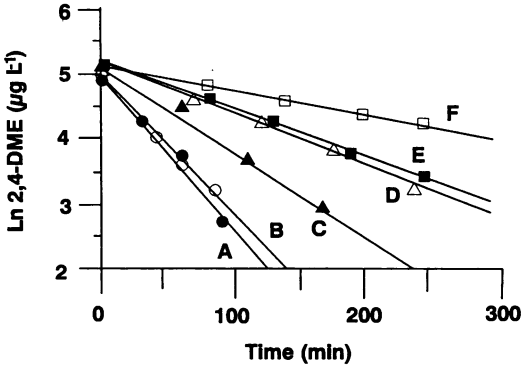

FIG. 1. Inhibitory effects of Zn^{2+} on pseudo-first-order 2,4-DME biodegradation rates by algae-dominated aufwuchs (floating mats). The slopes of regression lines of the natural logarithms of chemical concentration versus time equal the rate coefficients, k_1. Treatments shown are control (A) and 7.3 μM (B), 73 μM (C), 0.73 mM (D), 7.3 mM (E), and 73 mM (F) $ZnCl_2$.

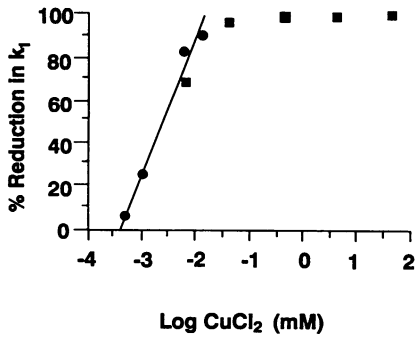

FIG. 2. Inhibitory effects of a wide range of $CuCl_2$ concentrations in aufwuchs on pseudo-first-order degradation rate coefficients, k_1. Data are from separate experiments using 0.5 μM to 0.05 mM and 5.9 μM to 59 mM $CuCl_2$.

molar concentrations of metals; (ii) metal concentrations that caused a significant effect on biodegradation parameters (both a >10% decrease in V_{max} and a >10% increase in the $t_{1/2}$ for 2,4-DME degradation); and (iii) HLDs for 2,4-DME biodegradation rates, which were based on the linear portions of plots of percent decrease in k_1 versus logarithms of molar concentrations of metals.

MICs varied according to metals and the type of microbial sample (sediment or aufwuchs) tested and did not necessarily follow the toxicity patterns observed for the metal concentrations required for significant effects on V_{max} and $t_{1/2}$. The MICs of metals in sediment were lowest for Zn^{2+} (0.10 μM) and highest for Cd^{2+} and Cu^{2+} (0.9 and 1.2 μM, respectively). The MICs of metals in aufwuchs were lowest for Hg^{2+} (0.01 μM), intermediate for Cu^{2+} and Zn^{2+} (0.42 and 0.62 μM, respectively), and highest for Cr^{3+} and Cd^{2+} (3.4 and 5.6 μM, respectively).

Generally, it required more than 7.0 μM Zn^{2+}, 25 μM Cr^{3+}, and 100 μM Cd^{2+} to cause both a >10% decrease in V_{max} and a >10% increase in the $t_{1/2}$ for 2,4-DME degradation. By using these criteria as a measure of significant effects, aufwuchs samples were considerably more sensitive to copper than sediment samples. More than 30 μM Cu^{2+} was required to significantly affect biodegradation in sediments, while only a 1.0 μM concentration of this metal significantly affected aufwuchs. Compared with Cu^{2+} on aufwuchs, 70 times more Zn^{2+}, 250 times more Cr^{3+}, and 1,000 times more Cd^{2+} was required to significantly affect aufwuchs biodegradation rate parameters and coefficients (V_{max}, $t_{1/2}$). Aufwuchs was significantly affected by the lowest Hg^{2+} concentration tested (5 μM). Inhibitory effects increased with increasingly higher initial concentrations of the metal salts. Compared with $ZnCl_2$ and $Cd(NO_3)_2$, $CuCl_2$ was generally more inhibitory to 2,4-DME biodegradation. As little as ca. 5 μM $CuCl_2$ reduced the V_{max} values for lake sediments and aufwuchs communities by factors of 0.94 and 0.32, respectively (ratio of treatment V_{max} to unamended culture V_{max}).

A consistent relationship between k_1 and metal ion concentrations in which k_1 decreased with higher initial metal concentrations was observed (Fig. 1). When percentage decreases in k_1 were plotted as a function of the logarithms of metal concentrations, a linear relationship was obtained for a range of the lowest metal concentrations (Fig. 2). The upper limit of percent inhibition that was within a linear response range ($r > 0.92$) for the biodegradation rate experiments was 83 ± 7.5 ($n = 8$). Because HLDs were within the linear range of observed inhibition levels, nonlinear regression analyses of the data were not required in this study. Nonlinear responses for higher metal concentrations may have resulted from metal salt precipitation, binding effects, or other interactions of the metals with organic or inorganic materials in the samples.

Percentage decreases in k_1 values plotted as a function of the logarithms of metal concentrations were subjected to linear regression analysis to estimate HLDs and compare correlation (r) and regression coefficients (slopes). These data are summarized in Table 2. Figure 3 is a typical regression plot for the inhibition data.

The HLDs of metals in sediment were 0.17, 0.24, and 0.79 mM for Cu^{2+}, Cd^{2+}, and Zn^{2+}, respectively. The HLDs of metals in aufwuchs were 2.0 and 3.0 μM for Cu^{2+} and Hg^{2+}, respectively, and 0.14, 0.44, and 1.2 mM for Cr^{3+}, Zn^{2+}, and Cd^{2+}, respectively.

Like MICs, HLD determinations also indicated that various metals caused different levels of inhibitory effects on biodegradation rates depending on the metal and type of microbial sample. Moreover, biodegradation rates were very sensitive to increases in the concentrations of some metals (Table 2). Consequently, the order of toxicity of a group of metals causing a 50% reduction in biodegradation rates

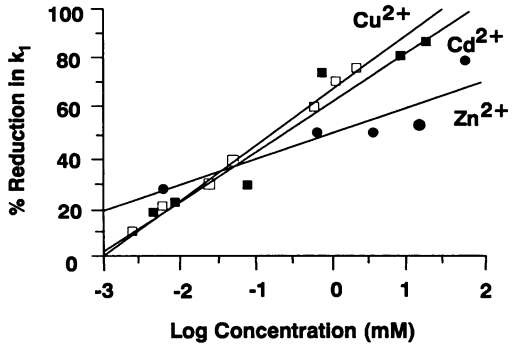

FIG. 3. Regression lines representing the effects of Cu^{2+}, Cd^{2+}, and Zn^{2+} concentrations in sediment on 2,4-DME biodegradation rate coefficients (k_1) relative to k_1 for samples unamended with metals.

TABLE 2. Concentrations of metal cations inhibiting 2,4-DME biodegradation in two experiments

Cation[a]	Microbiota	Slurry pH	Correlation coefficient (r)	Slope	MIC[b] (μM) (mean ± SD)	HLD[c] (mM) (mean ± SD)
Cu^{2+}	Sediment	6.1	1.0	23	1.2 ± 0.68	0.17 ± 0.008
	Aufwuchs	5.0	0.99	67	0.42 ± 0.045	0.002 ± 0.00009
Zn^{2+}	Sediment	6.4	0.97	11	0.097 ± 0.096	0.79 ± 0.36
	Aufwuchs	5.6	0.92	17	0.62 ± 0.05	0.44 ± 0.002
Hg^{2+}	Aufwuchs	6.8	0.98	26	0.012 ± 0.006	0.003 ± 0.0006
Cr^{3+}	Aufwuchs	6.1	0.96	34	3.4 ± 0.16	0.14 ± 0.01
Cd^{2+}	Sediment	6.5	0.97	21	0.89 ± 0.06	0.24 ± 0.01
	Aufwuchs	5.6	1.0	22	5.6 ± 0.25	1.2 ± 0.11

[a] Cu^{2+}, Hg^{2+}, and Zn^{2+} added as the chloride salts; Cr^{3+} and Cd^{2+} added as nitrates.
[b] MICs caused no reduction in k_1 values. Numerically, these values are abscissa intercepts (Fig. 2 to 4).
[c] HLD, concentration that caused half-life doubling.

differed from the order for the same metals causing a different percentage reduction in biodegradation rates.

Evidence that microbiota in sediment and aufwuchs samples responded differently to metal salt amendments was indicated by both HLDs and the slopes of the dose-response lines. Copper showed the most pronounced difference in responses between the two sources of microbiota. HLDs indicated that Cu^{2+} was about 85 times more inhibitory to 2,4-DME degradation by algae-dominated aufwuchs than by sediment microbiota (Table 2). Moreover, the slopes of regression lines for percentage inhibition of k_1 versus logarithms of metal cation concentrations indicated that aufwuchs samples were three times as sensitive as sediment samples to increases in copper concentrations (Table 2 and Fig. 4). According to published data on the effects of copper on algal cultures (5, 20), phytoplankton vary widely in their sensitivity to this metal. Therefore, cases in which the slopes for 2,4-DME degradation by aufwuchs microbiota (mainly filamentous algae) varied over a wide range of cupric ion concentrations (Fig. 2) may be explained as differences in biological properties (e.g., species composition, physiological state) of the populations responsible for 2,4-DME degradation. However, sediments may have had only a physical effect on the apparent toxicity of copper to microbial populations, i.e., may have sequestered more of the metal in a nonavailable phase, leaving less of it dissolved in the interstitial pore water occupied by microbes. Consequently, sediment populations in the absence of sediments may be as sensitive to copper as aufwuchs-associated microorganisms are.

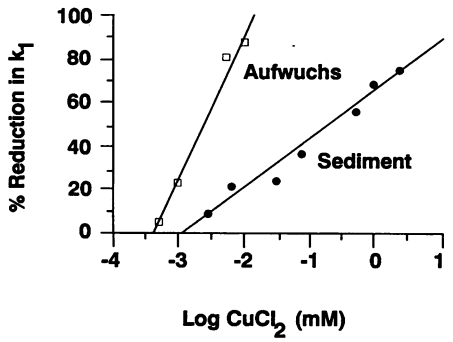

FIG. 4. Inhibitory effects of a wide range of $CuCl_2$ concentrations on sediment and aufwuchs microbiota.

When considering inhibition data, one must be careful to take into account the possible effects of various factors, such as pH, presence of chelators, and salinity. Because low pH increases the proportion of free ions in solution (16) for example, toxicity may be greater in acidic water than in basic water. On the other hand, metal toxicity is diminished in the presence of complexing substances. Chelated forms of heavy metals such as copper and cadmium, for example, are less toxic than the free ions. Therefore, relating inhibition results to dissociated metal concentrations may be needed for better correlations of metal concentrations with toxicity effects.

Even with improved correlations, however, the influence of different physical and chemical environmental characteristics on metal toxicity (16, 18, 19) may still present difficulties in developing an accurate quantitative relationship between metal concentrations and their inhibitory effects. Mathematical modeling approaches, such as MINTEQA2/PRODEFA2 (22) for predicting metal speciation in various environments, should be helpful when considering HLDs or other data in risk assessments.

Although this method could be modified to account for various environmental factors that might influence metal toxicity, the inhibition data clearly showed that even very low concentrations of some metals can severely retard biodegradation rates of an organic chemical. In previous studies (7), it was found that microbially toxic organics could inhibit biodegradation rates of those and other toxic organics. However, concentrations of organics in the range of 1 to 10 mM were generally required to severely inhibit biodegradation. Some of the heavy metals used in this study caused comparable biodegradation inhibition when present in concentrations 100- to 1,000-fold or more dilute than the microbially toxic organics previously studied. Therefore, the presence of even much lower concentrations of heavy metals in the environment may be an important factor influencing biodegradation rates of organic chemicals.

To better characterize the inhibition of biodegradation by toxic metals, additional studies are needed that incorporate a variety of benchmark organic chemicals and various manipulations of environmental factors that affect important factors such as metal speciation. This information will give an understanding of environmental situations in which biodeg-

radation is unexpectedly low, such as at land disposal sites where metals and metal-containing chemicals are codisposed of with organic matter. It will also enable regulatory guidelines to be established for codisposing of organic chemicals and other wastes with toxic metals.

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REFERENCES

1. **Barnhart, C. L., and R. Vestal.** 1983. Effect of environmental toxicant on metabolic activity of natural microbial communities. Appl. Environ. Microbiol. **46**:970–977.
2. **Bauer, J., and D. G. Capone.** 1985. Effect of four aromatic organic pollutants on microbial glucose metabolism and thymidine incorporation in marine sediments. Appl. Environ. Microbiol. **49**:828–835.
3. **Boney, A. D.** 1970. Sub-lethal effects of mercury on marine algae. Mar. Pollut. Bull. **2**:69–71.
4. **Capone, D. G., D. Reese, and R. P. Kiene.** 1983. Effects of metals on methanogenesis, sulfate reduction, carbon dioxide evolution, and microbial biomass in anoxic salt marsh sediments. Appl. Environ. Microbiol. **45**:1586–1591.
5. **Erickson, S. J., N. Lackie, and E. Maloney.** 1970. A screening technique for estimating copper toxicity to estuarine phytoplankton. J. Water Pollut. Control Fed. **42**:R270–R278.
6. **Forstner, V., and G. T. W. Wittmann.** 1981. Metal pollution in the aquatic environment. Springer-Verlag, Berlin.
7. **Hwang, H.-M., R. E. Hodson, and D. L. Lewis.** 1989. Assessing interactions of organic compounds during biodegradation of complex waste mixtures by naturally occurring bacterial assemblages. Environ. Toxicol. Chem. **8**:209–214.
8. **Jonas, R. B., C. G. Gilmour, D. L. Stoner, M. M. Weir, and J. H. Tuttle.** 1984. Comparison of methods to measure acute metal and organometal toxicity to natural aquatic microbial communities. Appl. Environ. Microbiol. **47**:1005–1011.
9. **Kiene, R. P., and D. G. Capone.** 1984. Effects of organic pollutants on methanogenesis, sulphate reduction, and carbon dioxide evolution in salt marsh sediments. Mar. Environ. Res. **13**:141–160.
10. **Lawrence, J. A., and E. M. Wilson.** 1974. Sub-lethal effects of several metallic salts-organic compounds combinations upon the heterotrophic microflora of a natural water. Water Res. **8**:101–105.
11. **Lewis, D. L., and D. K. Gattie.** 1988. Prediction of substrate removal rates of attached microorganisms and of relative contributions of attached and suspended communities at field sites. Appl. Environ. Microbiol. **54**:434–440.
12. **Lewis, D. L., and H. W. Holm.** 1981. Rates of transformation of methyl parathion and diethyl phthalate by aufwuchs microorganisms. Appl. Environ. Microbiol. **42**:698–703.
13. **Lewis, D. L., H. P. Kollig, and T. L. Hall.** 1983. Predicting 2,4-dichlorophenoxyacetic acid ester transformation rates in periphyton-dominated ecosystems. Appl. Environ. Microbiol. **46**:146–151.
14. **Lewis, D. L., J. D. Pope, D. K. Gattie, and W. A. Said.** 1989. Laboratory procedure for determining substrate removal rate coefficients for biofilms. J. Microbiol. Methods **10**:103–111.
15. **McIntyre, A. D., and C. F. Mills.** 1975. Ecological toxicology research: effects of heavy metal and organohalogen compounds. Plenum Publishing Corp., New York.
16. **Moore, J. W., and S. Ramamoorthy.** 1984. Heavy metals in natural waters: applied monitoring and impact assessment. Springer-Verlag, New York.
17. **Newton, T. D., D. K. Gattie, and D. L. Lewis.** 1990. Initial test of the benchmark chemical approach for predicting microbial transformation rates in aquatic environments. Appl. Environ. Microbiol. **56**:288–291.
18. **Saunda, W. G., and P. A. Gillespie.** 1979. The responses of a marine bacterium to cupric ion and its use to estimate cupric ion activity. J. Mar. Res. **37**:761–777.
19. **Saunda, W. G., and R. R. L. Guillard.** 1976. The relationship between cupric ion activity and the toxicity of copper to phytoplankton. J. Mar. Res. **34**:511–529.
20. **Thomas, W. H., and D. L. Seibert.** 1977. Effects of copper on the dominance and the diversity of algae: controlled ecosystem pollution experiment. Bull. Mar. Sci. **27**:23–33.
21. **U.S. Environmental Protection Agency.** 1979. Water-related environmental fate of 129 priority pollutants. EPA-440/4-79-029. U.S. Environmental Protection Agency, Washington, D.C.
22. **U.S. Environmental Protection Agency.** MINTEQA2/PRODEFA2, a geochemical assessment model for environmental systems: version 3.0 user's manual, in press. U.S. Environmental Protection Agency, Washington, D.C.
23. **Vives-Rego, J., D. Vaque, and J. Martinez.** 1986. Effect of heavy metals and surfactants on glucose metabolism, thymidine incorporation and exoproteolytic activity in sea water. Water Res. **20**:1411–1415.